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The Mechanism of Scutellarin on Pancreatic Cancer Cells was Studied Based on Network Pharmacology

Zixin Xu[1,a], Faping Song[1,b], Shuangquan Wang[1,2,c,*]

[1]Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712046, China
[2]The Third Affiliated Hospital of Shaanxi University of Chinese Medicine, Xi'an XD Group Hospital, Xi'an, Shaanxi, 710077, China
[a]445009147@qq.com, [b]710043936@qq.com, [c]13572970680@139.com
[*]Corresponding author

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Abstract: Objective: To explore the potential target and mechanism of action of scutellarin in the treatment of pancreatic cancer by network pharmacology. Methods: Swiss Target Prediction was used to extract the potential target of scutellarin. The targets of pancreatic cancer were obtained from Gene Cards and OMIM databases. R software was used to analyze and obtain common targets of drugs and diseases. Cytoscope3.7.2 software plug-in CtyoNCA topology was used to establish the protein interaction (PPI) network diagram using STRING database to analyze the key targets of drug therapy in disease. R software was used for GO enrichment analysis. The Metascape website was used for enrichment analysis of KEGG pathway to predict the signaling pathway of scutellarin in the treatment of pancreatic cancer. Results: A total of 91 targets related to scutellarin in the treatment of pancreatic cancer were screened, including TNF, EGFR, CASP3, HSP90AA1, HRAS, etc. By GO enrichment analysis, 932 items were obtained, including 797 BP, 58 CC, and 77 MF. Through KEGG enrichment analysis, we obtained 13 tumor-related KEGG pathways, including anti-folate resistance, C-type lectin receptor signaling pathway, nitrogen metabolism, galactose metabolism, cancer pathway, neural active ligand-receptor interaction, purine metabolism, etc. Conclusion: This study indicates that scutellarin can act on pancreatic cancer through multiple targets and pathways. Scutellarin has a potential therapeutic effects on pancreatic cancer. This study provides a new basis for curcumin in the treatment of pancreatic cancer.

Pancreatic Cancer (PC) is one of the most common malignant tumors in the digestive system. Due to its excessive onset and low rate of early diagnosis, pancreatic cancer tends to be at an advanced stage when clinical symptoms develop, and more than 85% of pancreatic cancer patients have local invasion or distant metastasis of cancer cells at the first diagnosis [1]. The median survival time of pancreatic cancer patients is only 5-6 months, and the 5-year survival rate is less than 5%[2], so pancreatic cancer is also known as the "king of cancer". In this study, the target and related signaling pathways of baicalin in the treatment of pancreatic cancer were predicted and analyzed by using network pharmacology technology, in order to provide ideas and experimental reference for the future research of TCM treatment of pancreatic cancer.

1. Data and Methods

1.1. Collect the Target of Scutellarin on Pancreatic Cancer

Use SwissTarget Prediction (http: //www.swisstargetprediction. ch /) to predict the potential target of scutellarin. The specific composition is as follows: PubChem (https: //pubchem. ncbi. nlm. nih. gov /) is a database about chemical modules. Query the relevant chemical information of scutellarin through PubChem database, and download its 2D compound structure file in SDF format. Upload the SDF file to the Swiss Target Prediction website, select "Homo sapiens" in the species selection column, click "Predict targets", and save the search results in xlsl format.

Targets related to pancreatic cancer were collected from GeneCards (https; //www.genecards. org /) and OMIM (https: //www.omim. org /). The specific process is as follows: Genecards is a comprehensive human gene database, providing information related to gene expression, gene function, protein mutations, transcription, genetics, etc. The full name of OMIM is "Online Mendelian Inheritance in Man". OMIM mainly includes human genes and genetic disorders. With "Pancreaticcancer" as the keyword, search the target of pancreatic cancer in GeneCards database and OMIM database, respectively. Use R software to merge the pancreatic cancer targets obtained from the two databases. The cross-analysis of scutellarin target and pancreatic cancer target was performed with R software, and the results were plotted as Venn diagrams.

1.2. Construction of Protein protein Interaction Network (PPI) and Screening of Core Targets

String database (https: //string-db. org /) is one of the databases with the most abundant data and the most widely used for studying protein interactions. Upload the target protein obtained in the first step to the string database, select the "multipleproteins" option, set the species to Homesapiens, set the minimum interaction score to 0.400, and keep the default value for others. Obtain the protein interaction diagram and save the data results in csv format. Upload the data results downloaded from the String website to Cytoscape 3.7.2, analyze the node degree of the network topology parameters through the built-in plug-in NetworkAnalyzer, and generate a visual network. The higher the degree value is, the wider the role of this node and other nodes are, and it plays a pivotal role in the network.

1.3. Gene Ontology (Go) Enrichment Analysis

GO is a functional system, which aims to clarify gene functions and attributes of gene products [3]. The R software was used to perform GO enrichment analysis on the intersection targets of diseases and drugs, including molecular function (MF), biological process (BP), and cellular component (CC). P< 0.05 showed that the difference was statistically significant. The above results are implemented by R language colorspace software package, string software package, Gg-plot2 software package, and clusterProfiler software package.

1.4. Enrichment Analysis of Kegg Pathway

Metascape (https://metascape.org/gp/index.html#/main/step1) is a powerful tool for gene function annotation analysis, which can help users to apply the current popular bioinformatics analysis methods to the analysis of batch genes and proteins, to realize the cognition of gene or protein functions. The intersection target of disease and drug was uploaded to Metascape, and H.sapiens was selected, and then KEGG pathway enrichment analysis was performed on the intersection target.

2. Result

2.1. Anti Pancreatic Cancer Target of Scutellarin

In GeneCards and OMIM data, 12834 genes were obtained as targets for pancreatic cancer after removing duplicate targets; with the help of Swiss Target Prediction database, 100 potential targets of scutellarin were retrieved, and 91 intersection targets were obtained after mapping with pancreatic cancer targets (Figure 1), suggesting that scutellarin may be a potential target for the treatment of pancreatic cancer.

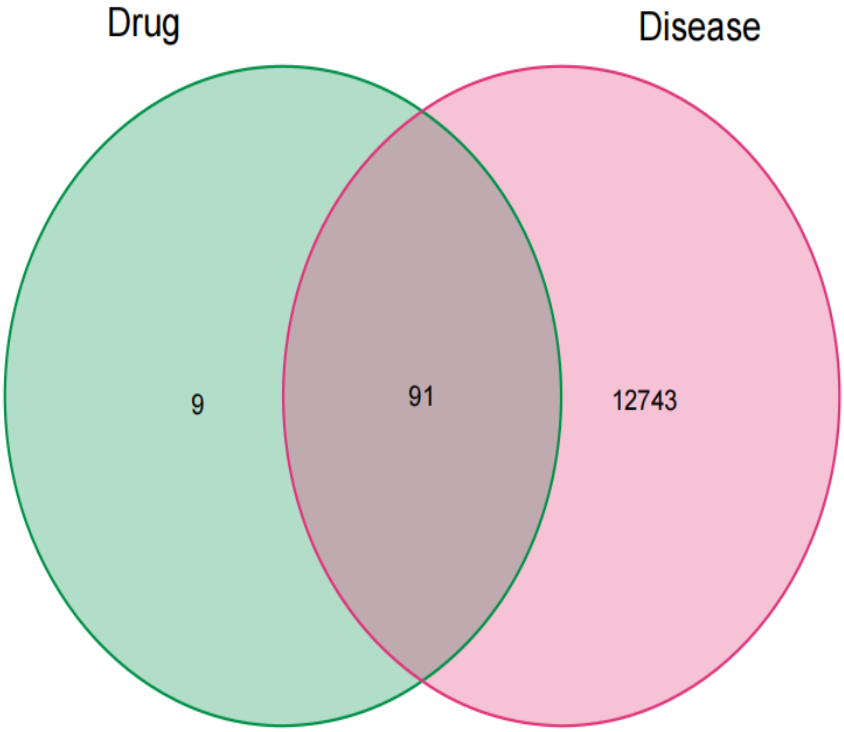


Figure 1: Venn diagram of drug and disease targets.

2.2. Construction of PPI Protein Interaction Network Diagram and Analysis of Core Targets

The PPI network diagram (Figure 2) has 91 nodes and 321 edges, that is, 321 protein interactions. Among them, green represents gene neighborhood, red represents gene fusion, and blue represents gene co-occurrence. The average node degree is 7.05, and the average local clustering coefficient is 0.528.

Use the Cytoscape software to generate the PPI network core target map (Figure 3). The color represents the size of the degree. The darker the color, the higher the ranking. The results showed that the top five targets were TNF, EGFR, CASP3, HSP90AA1, and HRAS, and the corresponding degree values were 35, 31, 27, 21 and 20 respectively. The analysis results indicate that the above targets may be the key proteins of scutellarin in the treatment of pancreatic cancer.

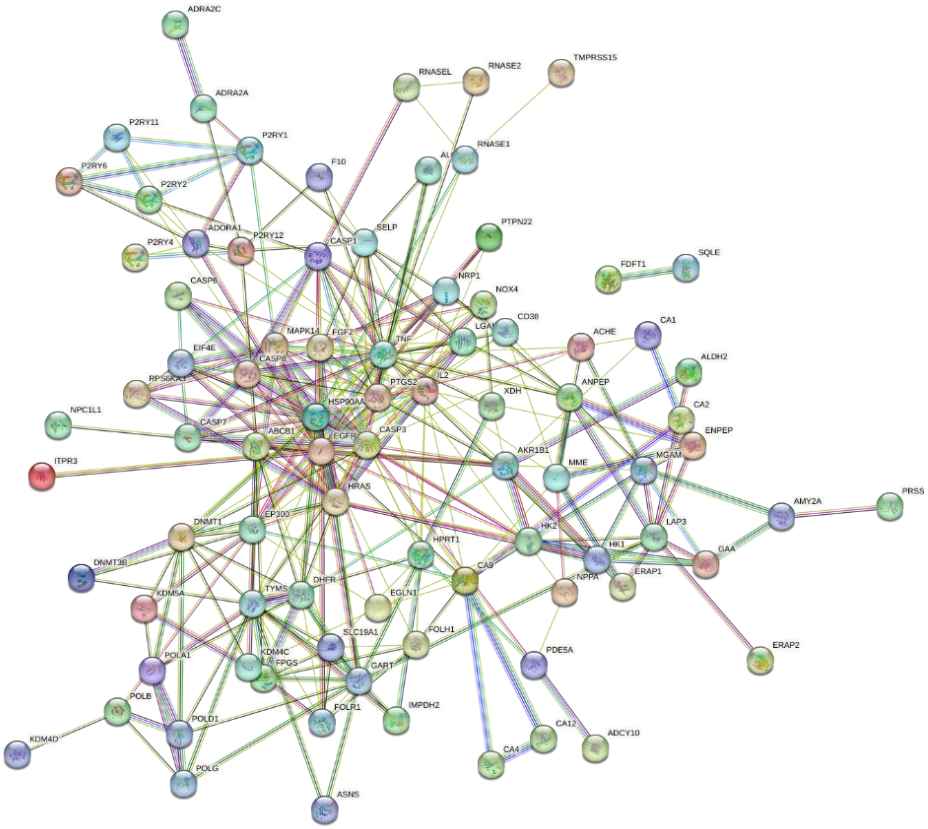


Figure 2: Protein interaction network.

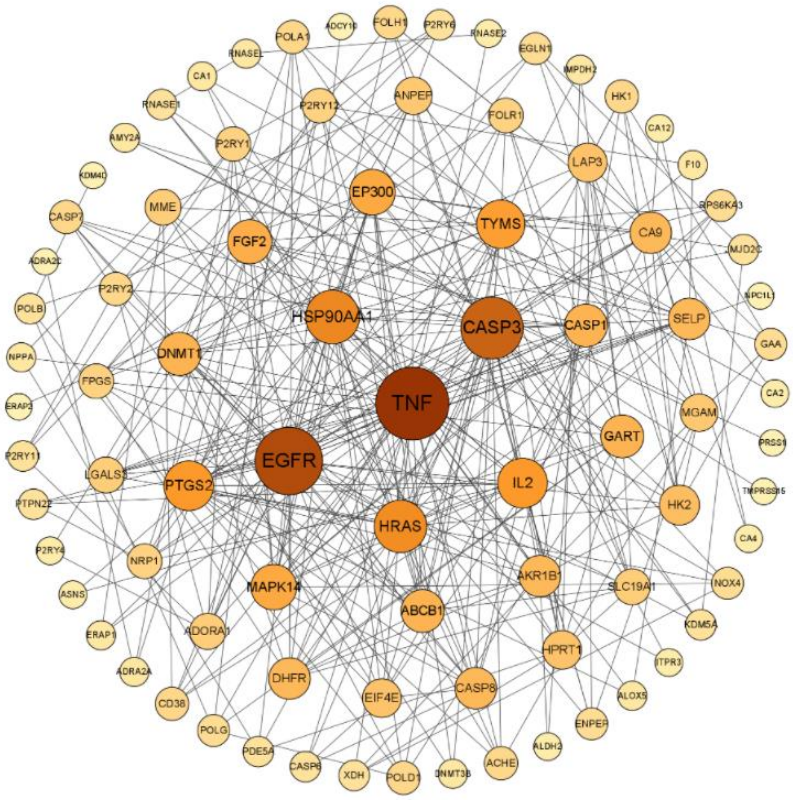
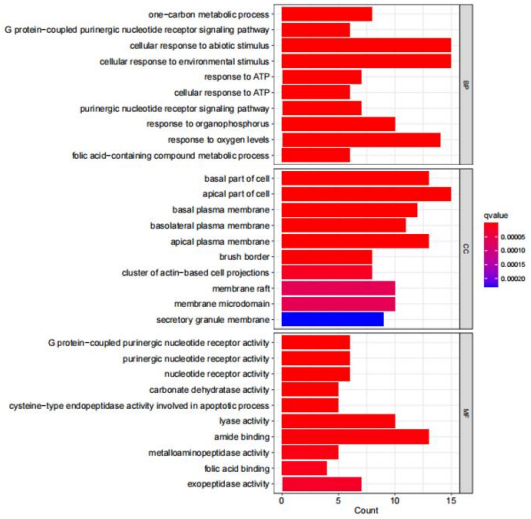


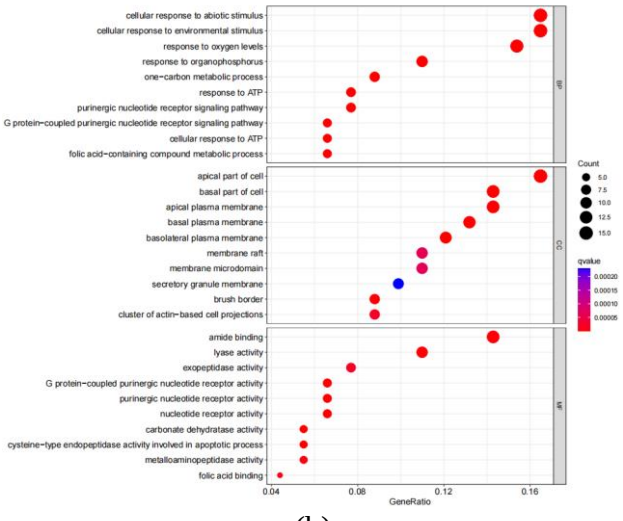
Figure 3: Core Target of PPI Network.

2.3. GO Enrichment Analysis Results

932 entries were obtained through GO function enrichment analysis, including 797 biological process (BP), 58 cellular components (CC), and 77 molecular functions (MF) (Figure 4). The results showed that scutellarin treatment mainly played a role in the treatment of pancreatic cancer in the basal part of cells, the apical part of cells, the basal plasma membrane, basolateral plasma membrane, apical plasma membrane, the brush border, the cluster of actin-based cell projections, the membrane raft, membrane microdomain, secretory granule membrane. The BPS involved in the treatment of pancreatic cancer with scutellarin mainly include: one-carbon metabolic process, G protein-coupled purinergic nucleotide receptor signaling pathway, cellular response to abiotic stimuli, cellular response to environmental stimuli, response to ATP, cellular response to ATP, purinergic nucleotide receptor signaling pathway, response to organophosphorus, response to oxygen levels, folic acid-containing compound metabolic process. It mainly affects the G protein-coupled purinergic nucleotide receptor activity, purinergic nucleotide receptor activity, nucleotide receptor activity, carbonate dehydratase activity, cysteine-type endopeptidase activity involved in apoptotic process, lyase activity, amide binding, metalloaminopeptidase activity, folic acid binding, exopeptidase activity.



(a)



(b)

Figure 4: GO enrichment analysis (a), (b).

2.4. Enrichment Results of KEGG Pathway

Through KEGG enrichment analysis, we obtained 13 tumor-related KEGG pathways (Figure 5). The signal pathways involved include Antifolate resistance, C-type lectin receptor signaling pathway, Nitrogen metabolism, Galactose metabolism, Pathways in cancer, Neuroactive ligand-receptor interaction, Purine metabolism, Coronavirus disease - COVID-19, cGMP-PKG signaling pathway, Pancreatic secretion, Serotonergic synapse, Renin-angiotensin system, Platelet activation, etc.

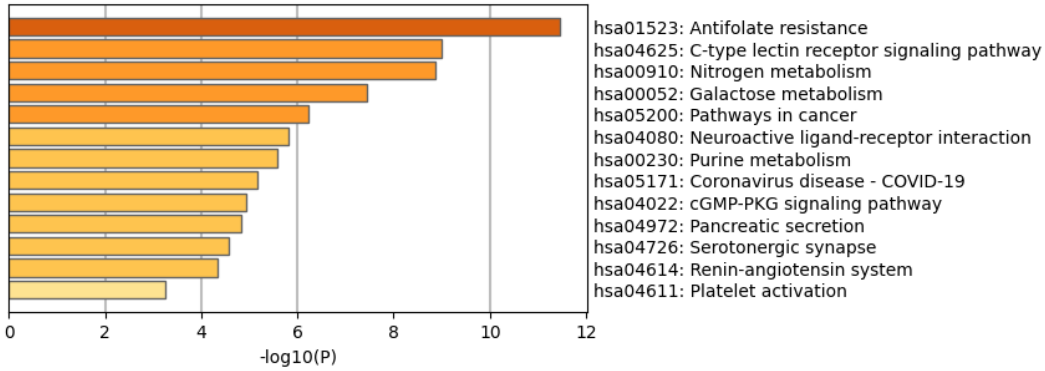


Figure 5: Enrichment map of scutellarin and tumor-related KGGG pathway.

3. Discuss

Pancreatic cancer is a common malignant tumor of the digestive system. For a long time, there is a lack of effective treatment for pancreatic cancer clinically, resulting in a similar incidence rate and mortality, and a poor prognosis [4]. Pancreatic cancer belongs to the category of "Fu Liang", "Ji Ju", "Zheng Jia" and other diseases in TCM theory. Some scholars believe that the pathogenesis of pancreatic cancer is mainly caused by dampness, toxin, heat, and congestion [5] [6]. Scutellarin is one of the main components of scutellaria baicalensis and scutellaria barbata. Scutellaria baicalensis has the effects of clearing away heat and dampness, purging fire, and detoxifying, while Scutellaria barbata has the effects of clearing away heat, detoxifying and removing blood stasis. It has been proved that scutellarin has a certain anticancer effect [7].

In this study, the potential target genes and possible signal pathways of the antitumor effect of scutellarin were mined and obtained through multiple software and databases using the method of network pharmacology. In this study, 91 common targets of scutellarin and pancreatic cancer were collected. The key targets of scutellarin acting on pancreatic cancer were screened through PPI protein interaction network, including TNF, EGFR, CASP3, HSP90AA1, HRAS, IL2, MAPK14, PTGS2, DNMT1, FGF2, EP300, TYMS, CASP1, GART, AKR1B1, ABCB1, etc. TNF, the full name of tumor necrosis factor, is a proinflammatory cytokine, which has a variety of biological effects and is closely related to the size and metastasis of tumor cells [8] [9]. EGFR may be the bridge of inflammation inducing tumor [10]. CASP3 is an important protein in the process of cell apoptosis, and activating CAPS3 protein can promote cell apoptosis [11]. HSP90AA1 is closely related to tumor cells. It is highly expressed in breast cancer tissue and is related to the survival period of patients [12].

The results of GO analysis showed that scutellarin mainly played a therapeutic role in the basal part of the cell, apical part of the cell, basal plasma membrane, basolateral plasma membrane, apical plasma membrane and other parts. The Mfs of scutellarin on pancreatic cancer mainly includes G protein-coupled purinergic nucleotide receptor activity, purinergic nucleotide receptor activity, nucleotide receptor activity, carbonate dehydratase activity, etc. Folate binding protein (FOLR1)

plays a essential role in cell division, proliferation, and growth and is closely related to drug resistance of tumor cells [13].

The BPS involved in the action of scutellarin on pancreatic cancer include one-carbon metabolic process, G protein-coupled purinergic nucleotide receptor signaling pathway, cellular response to abiotic stimuli, cellular response to environmental stimuli, response to ATP, etc. Research shows that metabolic disorder is closely related to the occurrence of cancer [14]. One-carbon metabolic process is an important process in cell metabolism. One-carbon metabolic process has three main reactions: folic acid cycle, methionine cycle, and reverse sulfurization pathway. In tumor cells, one-carbon metabolic process can promote tumor growth and inhibit tumor apoptosis [15].

The main pathways obtained by KEGG enrichment analysis include Antifolate resistance, C-type lectin receptor signaling pathway, Nitrogen metabolism, Galactose metabolism, Pathways in cancer, etc. Nitrogen metabolism is an important metabolic pathway. Research shows that nitrogen metabolism is closely related to the occurrence, invasion, and progress of cancer [16] [17] [18]. The main function of C-type lectin receptors is to activate and regulate the immune function of the host by recognizing specific ligands [19]. Studies have shown that C-type lectin receptor has great research value in tumor immunity. Type C lectin receptors can be used in the treatment of many cancers. Human DNGR-1 is one of the C-type lectin receptors. The use of specific antibodies and corresponding adjuvants of DNGR-1 can inhibit lung metastasis of melanoma [20] [21] [22].

To sum up, based on the methods and technologies of network pharmacology, this study screened the potential targets of scutellarin on human pancreatic cancer, with scutellarin and pancreatic cancer as the research objects, and studied the protein-protein interaction of the screened targets. At the same time, we also explored the possible mechanism of scutellarin in the treatment of pancreatic cancer. The study found that the treatment of pancreatic cancer with scutellarin is achieved through the joint coordination of multiple targets and pathways. The results showed that the effect of scutellarin on pancreatic cancer has a high research prospect. This study provides a certain idea for the follow-up research on the treatment of pancreatic cancer with scutellarin, and provides a preliminary basis for the research and development of new drugs based on scutellarin for pancreatic cancer. Computer simulation has some limitations, which can not completely replace experimental research. Therefore, the research on the therapeutic effect of scutellarin on pancreatic cancer also needs to be further verified by in vivo and in vitro experiments.

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